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The immature stages of the necrophagous fly *Liopiophila varipes* and considerations on the genus *Liopiophila* (Diptera: Piophilidae)

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Abstract

The preimaginal stages of *Liopiophila varipes* (Meigen, 1830) (Diptera: Piophilidae), the only species of the genus *Liopiophila* Duda, are described. The first and second-instar larvae and the puparium are described for the first time. The morphology of the third-instar larva is described in detail and compared with previous descriptions. Despite recent classifications suggested considering *Liopiophila* as a synonym of *Prochyliza* Walker, the presence of two rows of spines on the ventral creeping welts and fan-shaped anterior spiracles with lobes arranged in two groups in larvae and puparium support the validity of *Liopiophila* as a genus and its consideration as the sister group of the genus *Stearibia* Lioy. A key to the genera of the subtribe Piophilina based on the known larvae is provided.

Key Words

Larval morphology
Phylogeny
Forensic entomology
Stearibia nigriceps

Introduction

Widely distributed throughout the world but mainly represented in the cooler and temperate regions of the Northern Hemisphere (McAlpine 1977), the family Piophilidae is a small group of Diptera containing about 70 species (Ozerov 2004). Most of its species are scavengers; both adults and larvae can be frequently found on carcasses, preferably in advanced stages of decay (Martín-Vega 2011). Due to the attraction to proteinaceous animal matter and the synanthropic or hemisynanthropic habits of several species, they can represent economically important pests for the food industry (Zuska and Laštovka 1965).

Liopiophila varipes (Meigen, 1830) is a piophilid species with Holarctic distribution, frequently cited in association with carrion (e.g. Anton et al. 2011; Martín-Vega and Baz 2013). Despite *L. varipes* appears to be of smaller economic and hygienic importance than other piophilid species, it may be common on food industry premises (Zuska and Laštovka 1965). Moreover, apart from decaying animal matter, its larvae have also been recorded breeding on rotten leaves (Duda 1924). *Liopiophila* was described as a subgenus contained in genus *Piophila* Fallén by Duda (1924), a classification which was followed by subsequent specialists (e.g. Hennig 1943; Zuska and Laštovka 1965). Nevertheless, McAlpine (1977) ranked *Liopiophila* as a genus containing a single species, *L. varipes*, transferring the rest of species included by Duda (1924) in subgenus *Liopiophila* to the genus *Prochyliza* Walker. On the other hand, Ozerov (2004) considered *Liopiophila* as a synonym of *Prochyliza*, including *L. varipes* in the latter genus.

The morphological descriptions of the immature stages of insects are necessary for the correct identification of species in those cases in which it is not possible to rear to adulthood. In the case of necrophagous insects, a reliable identification of the immature stages is essential from a forensic point of view. The larval morphology of most piophilid species remains unknown (Martín-Vega 2011) but curiously the mature larva of *L. varipes* has been described twice (Nielsen et al. 1954; Brindle 1965), although both descriptions contain controversial char-

acters. The current paper describes the morphology of the immature stages of *L. varipes*, providing simple diagnostic characters allowing for its differentiation from other Piophilidae species and comparing the characters of third-instar larva with those previous descriptions. Some considerations on the status of genus *Liopiophila* based on larval morphology are given.

Material and methods

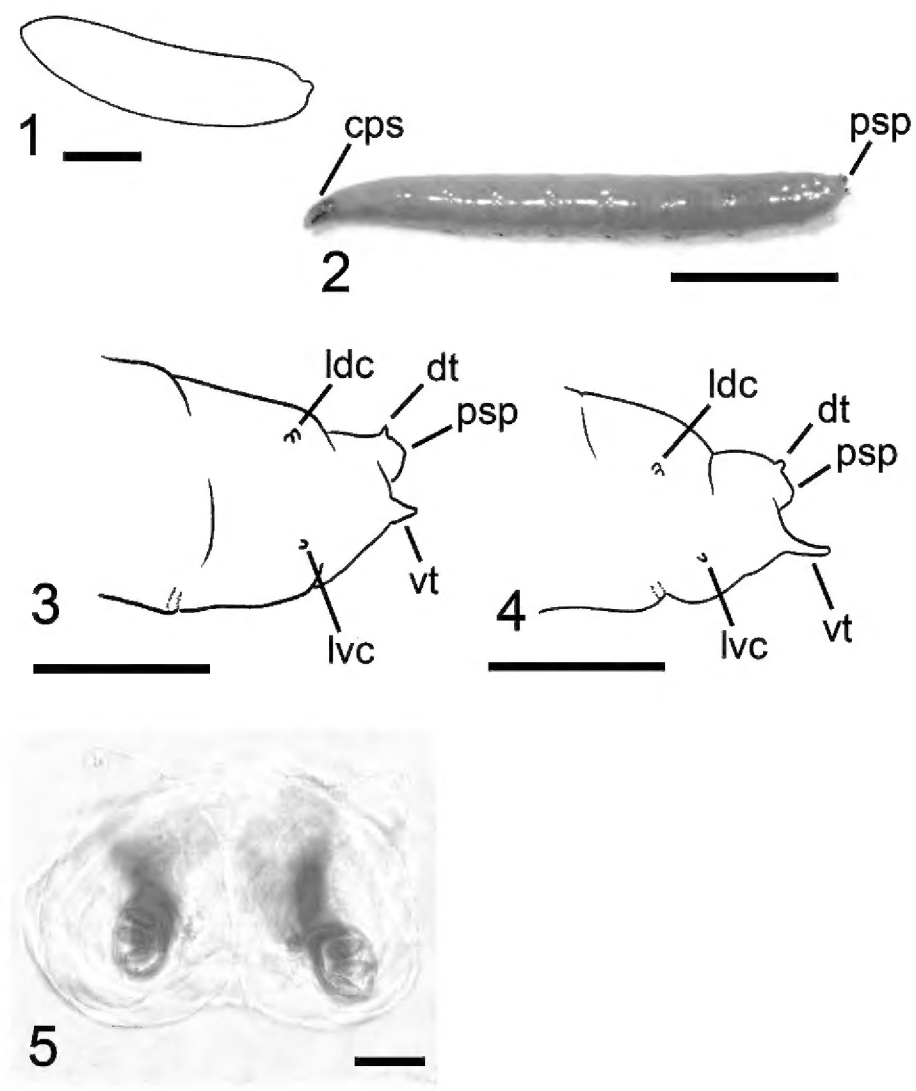
Adult flies of *L. varipes* were collected using pig carrion baits in a pine forest located in Puerto de Navafría (Madrid Province, central Spain) at 1810 m a.s.l. The flies were transferred to a plastic box of 230 × 155 × 115 mm with a gauze mesh at one side, and maintained at constant temperature (20 °C) and light photoperiod (12:12 h) inside a rearing camera. The flies were provided with water and granulated sugar cubes, as well as beef pâté which served as protein uptake to allow egg maturation and as substrate for oviposition. Plastic boxes were examined twice per day to trace whether females had spontaneously oviposited. Eggs were transferred to separate plastic tubs also containing beef pâté to feed emerging larvae, and sand to allow the burial and pupariation of post-feeding larvae. Plastic tubs were maintained in the same rearing camera with the adult colony.

First-instar larvae (L1), second-instar larvae (L2) and third-instar larvae (L3) were removed from the plastic tubs, killed in near-boiling water and then preserved in 80% ethanol. Twenty five L3 were measured within the first hour after killing and then preserved in 80% ethanol. Such killing and measuring procedure is recommended to minimize postmortem changes in larval length (Adams and Hall 2003). Twenty five eggs and puparia were also removed from the plastic tubs, directly measured and then preserved in 80% ethanol. Measurements of eggs were made under a light microscope equipped with a calibrated eyepiece micrometer; measurements of L3 and puparia were made under a binocular microscope with a micrometer and reticulated paper at ×8 magnification. L3 were clarified in hot 10% lactic acid, dissected and embedded in dimethyl hydantoin formaldehyde to study their different parts under a light microscope equipped with a camera lucida for drawings. Terminology follows Courtney et al. (2000) and Grzywacz et al. (2012). The studied material has been deposited in the collection of the Department of Life Sciences of the University of Alcalá.

Results

Egg

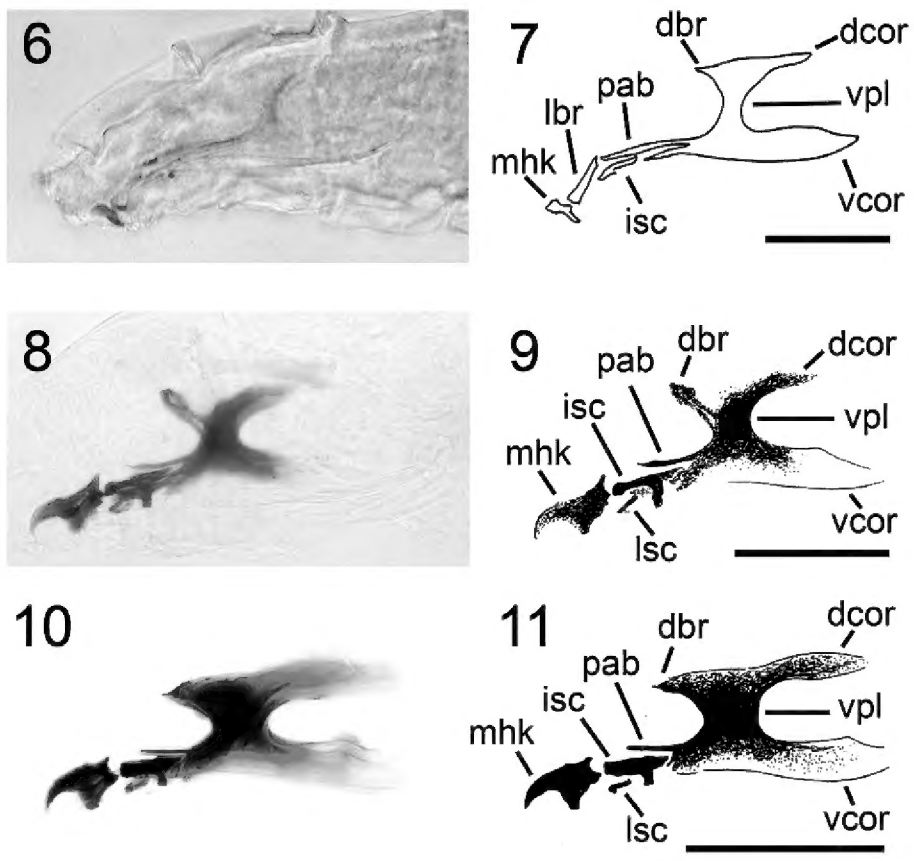
The eggs of *L. varipes* are white and long (mean length ± SD = 0.73 ± 0.03 mm; n = 25; range: 0.66–0.79), banana-shaped, with protuberant micropyle (Fig. 1), identical in general appearance to the eggs of other piophilid species (e.g. Hennig 1943, Martín-Vega et al. 2012).



Figures 1–5. Immature stages of *Liopiophila varipes* (Meigen) and *Stearibia nigriceps* (Meigen). **1.** Egg of *L. varipes*, scale bar 0.01 mm; **2.** Third-instar larva of *L. varipes*, scale bar 1.5 mm; **cps** – cephalopharyngeal skeleton; **psp** – posterior spiracle; **3.** Anal division of third-instar larva of *L. varipes*, lateral view, scale bar 0.5 mm; **4.** Anal division of third-instar larva of *S. nigriceps*, lateral view, scale bar 0.5 mm; **dt** – dorsal tubercles; **ldc** – laterodorsal cone; **lvc** – lateroventral cone; **psp** – posterior spiracle; **vt** – ventral tubercle; **5.** Posterior spiracles of *L. varipes*, scale bar 0.08 mm.

Larva

The larvae of *L. varipes* show the typical morphology of the piophilid larvae; the body is cylindrical, tapering gradually forwards, narrowing slightly backwards and then somewhat truncated (Fig. 2). The mean length ± SD of L3 was 7.14 ± 0.42 mm (n = 25; range: 6.30–7.70). The body is divided in 12 segments (pseudocephalon, three thoracic segments, seven abdominal segments, and anal division); the anal division shows the typical morphology of piophilid larvae with a pair of dorsal tubercles and a longer pair of ventral tubercles (Figs 3, 4). A pair of posterior spiracles is placed on fleshy prominences below the dorsal tubercles. The posterior spiracles show slit-like openings (two openings in L1 and three openings in L2 and L3) on a sclerotized plate surrounded by the peritreme (Fig. 5), as typically described for Cyclorrhapha larvae (Courtney et al. 2000). L3 showed the typical skipping behaviour observed in other species of Piophilidae by arching its body until the mouth hooks contact the anal tubercles, pulling and suddenly releasing them. The eleventh segment (i.e. seventh abdominal segment) shows two small pairs of laterodorsal and a small pair of lateroventral cones (Fig. 3).

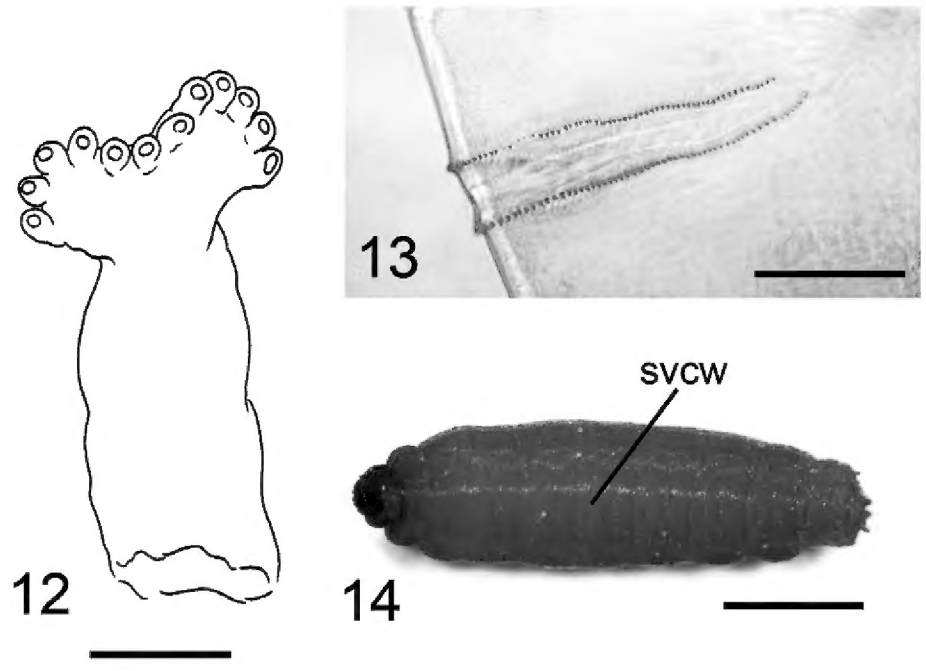


Figures 6–11. Cephalopharyngeal skeleton (CPS) of *Liopiophila varipes* (Meigen) larva. **6. 7** CPS of first-instar larva, scale bar 0.005 mm; **8, 9** CPS of second-instar larva, scale bar 0.1 mm; **10, 11** CPS of third-instar larva, scale bar 0.25 mm; **dbr** – dorsal bridge; **dcor** – dorsal cornua; **isc** – intermediate sclerite; **lbr** – labrum; **lsc** – labial sclerite; **mhk** – mouth hook; **pab** – parastomal bar; **vcor** – ventral cornua; **vpl** – vertical plate.

Pseudocephalon is bilobed and each lobe shows antennal organ and maxillary palpus as described by Courtney et al. (2000); antennal organs act as mechanoreceptors and olfactory receptors (Huckesfeld et al. 2010). The oral cavity shows a facial mask with oral comb plates surrounding the tips of the mouth hooks of cephalopharyngeal skeleton (hereafter CPS). In L1, the CPS is barely distinguishable, weakly sclerotized (Figs 6, 7) and very different from those of L2 and L3. In L2, the CPS is not completely sclerotized but clearly distinguishable (Figs 8, 9). The CPS of L3 is very similar to those of L2; its parts are well sclerotized and developed (Figs 10, 11). The dorsal edge of the CPS basal sclerite is convex, the dorsal and ventral cornua are long, and the dorsal bridge shows a broad base. The mouth hooks show a large base and their dorsal edge is slightly concave in its basal part (Figs 10, 11).

The first thoracic segment of L2 and L3 show a pair of anterior spiracles with fan-shaped arranged lobes at their distal edge (Fig. 12). The most frequent number of lobes of the anterior spiracles of L3 ranged from 10 to 12 (n = 10); the lobes are arranged in two groups (of five or six, respectively) (Fig. 12). In L1, anterior spiracles were not distinguishable under light microscope; however, a pair of simple and minute prothoracic spiracles has been observed in the L1 of a range of Diptera Cyclorrhapha families under scanning electron microscope (see Grzywacz et al. 2012).

The abdominal segments of the larvae show ventral creeping welts equipped with two rows of spines (Fig. 13). The spines of the ventral creeping welts and the mouth hooks grip the substrate making possible the advance of the larvae into dead tissues (Roberts 1971).



Figures 12–14. Immature stages of *Liopiophila varipes* (Meigen). **12.** Anterior spiracle, scale bar 0.05 mm; **13.** Rows of spines on the ventral creeping welts, scale bar 0.15 mm; **14.** Puparium, ventral view, scale bar 1 mm; **svcw** – spines of the ventral creeping welts.

Puparium

The puparium of *L. varipes* is barrel-shaped, brown to coppery red in colour (Fig. 14). The mean length ± SD of the puparia was 4.16 ± 0.15 mm (n = 25; range: 3.8–4.4). Because the puparium of cyclorrhaphous flies is formed from the cuticle of L3 (Fraenkel and Bhaskaran 1973), cuticular features of the larvae, including the two rows of spines on the ventral creeping welts, can be observed in the puparia. Also, the CPS of L3 can be extracted from empty puparia and displayed.

Discussion

Taking into account the economical interest of the larvae of the species of family Piophilidae (Zuska and Laštovka 1965), as well as the importance of careful and reliable identifications for their use in forensic entomology (Martín-Vega 2011), a complete larval identification key to this family would be desirable. A larval identification key to the genera of subtribe Piophilina is suggested below, following the classification of McAlpine (1977) and based on previous descriptions (Hennig 1943; McAlpine 1977; Ozerov 2000; Martín-Vega et al. 2012). Some characters from the key can be also observed on the puparia. Some characters need to be confirmed in genera *Arctopiophila* Duda, *Parapiophila* McAlpine and *Protopiophila* Duda; moreover, the considerations of Ozerov (2004) on the classification and validity of genera *Arctopiophila* and *Parapiophila* must be taken into account. For a general larval identification key to sarcosaprophagous Diptera families including Piophilidae see Velásquez et al. (2010). As mentioned, the larvae of most of the piophilid species remain undescribed and the scarce published descriptions are dispersed in the scientific literature, needing a compilation and updating. Further steps in these directions should be done.

Key to the larvae of subtribe Piophilina *sensu* McAlpine (1977)

1 Ventral anal tubercles very short, apparently equal in length to the dorsal anal tubercles. Anterior spiracles with 6 lobes (unknown in *Arctopiophila*). Ventral creeping welts equipped with 6 rows of spines (unknown in *Arctopiophila* and *Protopiophila*). See Hennig (1943) for details on *Parapiophila vulgaris* (Fallén), McAlpine (1977) for details on *Arctopiophila arctica* (Holmgren), and Ozerov (2000) for details on *Protopiophila latipes* (Meigen) ..... *Arctopiophila* Duda (2 spp.)
Parapiophila McAlpine (15 spp.)
Protopiophila Duda (11 spp.)

– Distinct combination of characters. Ventral creeping welts equipped with 2-3 rows of spines 2

2 Ventral creeping welts equipped with 3 rows of spines 3

– Ventral creeping welts equipped with 2 rows of spines 4

3 Ventral anal tubercles slightly directed ventrally. Dorsal edge of mouth hook slightly convex in its basal part. Distance between the base and the tips of the mouth hook approximately equal than the width of the mouth hook base. See Martín-Vega et al. (2012) for details on *Prochyliza nigrimana* (Meigen) ..... *Prochyliza* Walker (8 spp.)

– Ventral anal tubercles slightly directed posteriorly. Dorsal edge of the mouth hook slightly concave in its basal part. Distance between the base and the tips of the mouth hook approximately 1.3 times longer than the width of the mouth hook base. See Hennig (1943) and Martín-Vega et al. (2012) for details on *Piophila casei* (L.) ..... *Piophila* Fallén (2 spp.)

4 Anterior spiracles with the lobes arranged in a single group. See McAlpine (1977) for details on *Lasiopiophila pilosa* ..... *Lasiopiophila* Duda (1 sp.)

– Anterior spiracles with the lobes arranged in two groups 5

5 Ventral anal tubercles elongated (Fig. 4). See Hennig (1943) for details on *Stearibia nigriceps* ..... *Stearibia* Lioy (1 sp.)

– Ventral anal tubercles not elongated (Fig. 3). Cephalopharyngeal skeleton as in Figs 10 and 11 ..... *Liopiophila* Duda (1 sp.)

Identification of the larva described by Nielsen et al. (1954) as *L. varipes*

The current description of *L. varipes* L3 fits with the description provided by Nielsen et al. (1954) for larvae collected on the bones of a whale. The morphology of the CPS of the L3 figured by Nielsen et al. (1954), with the convex dorsal edge of the basal plate, the broad base of the dorsal bridge, and the large base of mouth hooks, strongly resembles the CPS of *L. varipes* (Figs 10, 11). Moreover, Nielsen et al. (1954) figured a fan-shaped anterior spiracle with ten lobes arranged in two groups of five, as well as two rows of spines on the ventral creeping welts; these characters match with those observed in the current study (Figs 12, 13). Nielsen et al. (1954) suggested that the described larva may belong to *L. varipes* or to *Prochyliza lundbecki* (Duda), justifying their decision in the presence of two rows of ventral creeping welts which 'should, according to the key in Hennig (1943), belong to the subgenus *Liopiophila*' (sic). However, in his larval identification key, Hennig (1943) differentiated the '*Piophila-Liopiophila* group' by the presence of three rows of spines on the ventral creeping welts. Hennig (1943) justified such affirmation in the characters of the larva of *Piophila casei* (L.). Hence, the identification of the described larva as *L. varipes* by Nielsen et al. (1954) was very likely due to a misinterpretation of the key of Hennig (1943), but paradoxically they appeared to be right in their decision.

Spiracles as diagnostic character for identifying *L. varipes* larvae

In his larval identification key, Brindle (1965) suggested as diagnostic characters of *L. varipes* the fan-shaped anterior spiracles with six lobes arranged in a single group and the pair of ventral anal tubercles directed ventrally. The orientation of the anal tubercles is a character which should be taken with caution because differences in this sense are not always easily distinguishable (Martín-Vega et al. 2012). On the other hand, the anterior spiracle figured by Brindle (1965) does not fit with the description of Nielsen et al. (1954) and with the current observations (Fig. 12). The larvae of most piophilid species show fan-shaped anterior spiracles with the lobes arranged in a single group (Hennig 1943; Martín-Vega et al. 2012). Furthermore, the lobes of the anterior spiracles of *L. varipes* are thick, crowded together in the distal, fan-shaped part of the anterior spiracle (Fig. 12). Such character was also noted by Nielsen et al. (1954). The anterior spiracles of the larvae of other piophilid species show different appearance; the lobes are thin and arranged more separately from each other (Hennig 1943; Martín-Vega et al. 2012).

Validity of the genus *Liopiophila* and systematics of Piophilidae

McAlpine (1977) suggested a phylogeny of the Piophilidae where the genera *Lasiopiophila* Duda, *Liopiophila*, *Stearibia* Lioy, *Piophila*, and *Prochyliza* form a monophyletic group sharing the following characters: a weak or absent outer postpronotal bristle, the male abdomen sternites divided and the seventh male sternite frequently bearing peg-like processes. Moreover, the four latter genera share the loss of outer postpronotal bristle and the reduction of hairiness, with *Liopiophila* being the sister group of *Stearibia*, and *Piophila* the sister group of *Prochyliza* (McAlpine 1977). McAlpine (1977) con-

sidered *Piophila* and *Prochyliza* as sister groups because of the presence of secondary male characters, but no apomorphy was suggested for the group formed by *Liopiophila* and *Stearibia*. Nevertheless, McAlpine (1977) described the genus *Liopiophila* as very similar in most respects to *Stearibia*, with the presence of setae on the anepimeron being the differential character of *Liopiophila*. In his fine discussion on the classification of the Piophilidae, Ozerov (2004) recognized this character as a unique characteristic of *L. varipes*, but he did not consider it sufficient to distinguish this species as a separated genus and suggested including it in the genus *Prochyliza*. The suggestion of Ozerov (2004) was based on the presence of a silvery-white strip of pubescence on the gena as characteristic of all those species.

The larval morphology of *L. varipes* supports, however, the phylogeny and classification suggested by McAlpine (1977), and therefore the validity of genus *Liopiophila*. The arrangement of thick lobes of the anterior spiracles in two groups is characteristic of the larvae of *L. varipes* (Fig. 12), but is identical in the larvae of *Stearibia nigriceps* (Meigen) (Hennig 1943). It must be mentioned that Hennig (1943) described the morphology of the CPS and anterior spiracles of *S. nigriceps* (named as *Piophila foveolata* Meigen) larvae from the characters observed on a puparium of this species, highlighting that the number of rows of spines on the ventral creeping welts could not be confirmed from such a specimen. The first author of the current manuscript has studied some larvae of *S. nigriceps* collected in a carrion-succession study (see Anton et al. 2011) which showed two rows of spines on the ventral creeping welts. The presence of two rows of spines on the ventral creeping welts is therefore a common character of both *L. varipes* and *S. nigriceps*, but it is also shown by the larva of *Lasiopiophila pilosa* (Staeger), which conversely show anterior spiracles with lobes arranged in a single group (McAlpine 1977). In the group formed by *Piophila* and *Prochyliza* the lobes of the anterior spiracles are also arranged in a single group (Hennig 1943; Martín-Vega et al. 2012), but with the ventral creeping welts being equipped with three rows of spines (Hennig 1943; Martín-Vega et al. 2012). Finally, the larvae of *L. varipes* and *S. nigriceps* can be differentiated by the morphology of the pair of ventral tubercles, which are distinctly more elongate in *S. nigriceps* (Figs 3, 4).

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References

Adams ZJO, Hall MJR (2003) Methods used for the killing and preservation of blowfly larvae, and their effect on post-mortem larval length. Forensic Science International 138: 50–61. doi: 10.1016/j.forsciint.2003.08.010

Anton E, Niederegger S, Beutel RG (2011) Beetles and flies collected on pig carrion in an experimental setting in Thuringia and their forensic implications. Medical and Veterinary Entomology 25: 353–364. doi: 10.1111/j.1365-2915.2011.00975.x

Brindle A (1965) Taxonomic notes on the larvae of British Diptera No. 21-the Piophilidae. The Entomologist 78: 158–160.

Courtney GW, Sinclair BJ, Meier R (2000) Morphology and terminology of Diptera larvae. In: Papp L, Darvas B (Eds) Contribution to a manual of Palaearctic Diptera (with special reference to flies of economic importance), vol. 1. Science Herald Press, Budapest, 85–161.

Duda O (1924) Revision der europäischen u. grönländischen sowie einiger südostasiat. Arten der Gattung Piophila Fallén (Dipteren). Konowia 3: 97–113, 153–203.

Fraenkel G, Bhaskaran G (1973) Pupariation and pupation in cyclorrhaphous flies (Diptera): Terminology and interpretation. Annals of the Entomological Society of America 66: 418–422.

Grzywacz A, Pape T, Szpila K (2012) Larval morphology of the lesser housefly, *Fannia canicularis*. Medical and Veterinary Entomology 26: 70–82. doi: 10.1111/j.1365-2915.2011.00968.x

Hennig W (1943) Piophilidae. In: Lindner E (Ed) Die Fliegen der Palaearktischen Region, vol. 5. Schweizerbart, Stuttgart, 1–52.

Huckesfeld S, Niederegger S, Heinzel HG, Spieß R (2010) The cephalic and pharyngeal sense organs of *Calliphora vicina* 3rd instar are mechanosensitive but have no profound effect on ongoing feeding related motor patterns. Journal of Insect Physiology 56: 1530–1541. doi: 10.1016/j.jinsphys.2010.05.004

Martín-Vega D (2011) Skipping clues: Forensic importance of the family Piophilidae. Forensic Science International 212: 1–5. doi: 10.1016/j.forsciint.2011.06.016

Martín-Vega D, Baz A (2013) Sarcosaprophagous Diptera assemblages in natural habitats in central Spain: spatial and seasonal changes in composition. Medical and Veterinary Entomology 27: 64–76. doi: 10.1111/j.1365-2915.2012.01028.x

Martín-Vega D, Baz A, Díaz-Aranda LM (2012) The immature stages of the necrophagous fly, *Prochyliza nigrimana*: comparison with *Piophila casei* and medicolegal considerations (Diptera: Piophilidae). Parasitology Research 111: 1127–1135. doi: 10.1007/s00436-012-2943-5

McAlpine JF (1977) A revised classification of the Piophilidae, including 'Neottiophilidae' and 'Thyreophoridae' (Diptera: Schizophora). Memoirs of the Entomological Society of Canada 103: 1–66. doi: 10.4039/entm109103fv

Nielsen P, Ringdahl O, Tuxen SL (1954) Diptera 1 (exclusive of Ceratopogonidae and Chironomidae). The Zoology of Iceland 3: 1–189.

Ozerov AL (2000) Family Piophilidae. In: Papp L, Darvas B (Eds) Contribution to a manual of Palaearctic Diptera (with special reference to flies of economic importance), Appendix. Science Herald Press, Budapest, 355–365.

Ozerov AL (2004) On the classification of the family Piophilidae. Entomological Review 84: 600–608.

Roberts MJ (1971) On the locomotion of cyclorrhaphan maggots (Diptera). Journal of Natural History 5: 583–590. doi: 10.1080/00222937100770421

Velásquez Y, Magaña C, Martínez-Sánchez A, Rojo S (2010) Diptera of forensic importance in the Iberian Peninsula: larval identification key. Medical and Veterinary Entomology 24: 293–308.

Zuska J, Laštovka P (1965) A review of the Czechoslovak species of the family Piophilidae with special reference to their importance to food industry (Diptera, Acalyptrata). Acta Entomologica Bohemoslovaca 62: 141–157.